normal, and no increase in intracellular release of trichocysts was observed. Although no information is yet available for *Paramecium*, freeze fracture micrographs of ionophore-induced mucocyst discharge in *Tetrahymena* (*10*) indicates that discharge occurs via the normal sequence of membrane fusion processes.

We envision the sequence as follows. When the cell is stimulated at its external surface, a transmembrane event at the site of the fusion rosette is triggered. The simplest explanation of this event would be that the rosette particles act as Ca^{2+} channels that open upon stimulation. A site-specific increase in Ca^{2+} between the two competent partner membranes would then induce membrane fusion and subsequent exocytosis (*11*). At 18°C in nd9 cells, in the presence of Ca^{2+} picric acid would stimulate exocytosis. In the presence of external Mg^{2+} rather than Ca^{2+}, no Ca^{2+} would be available and exocytosis would be blocked. At 27°C, where the fusion rosette is missing, with an external stimulus (that is, picric acid) exocytosis cannot take place even where Ca^{2+} is present in the medium. Ionophore addition would substitute for the rosette by providing the necessary Ca^{2+} pathway across the membrane.

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References and Notes

1. B. Satir, C. Schooley, P. Satir, *Nature (London)* **235**, 53 (1972).
2. ———, *J. Cell Biol.* **56**, 153 (1973).
3. B. Satir, *Symp. Soc. Exp. Biol.* **28**, 399 (1974); F. Wunderlich and V. Speth, *J. Ultrastruct. Res.* **41**, 258 (1972).
4. J. Beisson *et al.*, *J. Cell Biol.* **69**, 126 (1976).
5. R. Janisch, *J. Protozool.* **19**, 470 (1972).
6. W. W. Douglass, *Biochem. Soc. Symp.* **39**, 1 (1974); R. P. Rubin, *Calcium and the Secretory Process* (Plenum, New York, 1974), pp. 111–116.
7. H. Plattner, *Nature (London)* **252**, 722 (1974); ——— and S. Fuchs, *J. Histochem.* **45**, 23 (1975).
8. G. Fisher, E. S. Kaneshiro, P. D. Peters, *J. Cell Biol.* **69**, 429 (1976).
9. *Paramecium* has approximately 4000 mature positioned trichocysts. We lose approximately half of the discharged trichocysts during preparation for counting (experimentally determined), and we count discharge over only about 25 percent of the total cell surface. Therefore, our counts as shown represent about one-eighth of the true total trichocysts discharged, and a count of about 200 (Table 1) is equivalent to about 40 percent of total trichocysts in the cell.
10. S. L. Wissig and B. Satir, *J. Cell Biol.* **70**, 172a (1976).
11. Q. F. Ahkong, D. Fisher, W. Tampion, J. A. Lucy, *Nature (London)* **253**, 194 (1975); B. Satir, *J. Supramol. Struct.* **5**, 381 (1976).
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Double-Stranded RNA Is Present in Extracts of Tobacco Plants Infected with Tobacco Mosaic Virus

Abstract. *Serologically specific electron microscopy was used to detect double-stranded RNA in extracts of tobacco infected with tobacco mosaic virus. Assays were made immediately after extraction, without purification, concentration, or treatment with phenol or detergent. This indicates that the double-stranded RNA is native and is not an artifact induced by purification methods.*

The possible presence of double-stranded RNA in tissues infected with viruses that have single-stranded RNA genomes has been reviewed (*1*). RNA complementary to parental viral RNA is present in these tissues and is considered to function as a template for the synthesis of viral RNA. Evidence has been given to show that these complementary strands of RNA are double-stranded and, in contradiction, they are probably only loosely bound in short segments at the point of synthesis. The case for double-stranded RNA is supported by experiments showing that it can be isolated from virus-infected tissue and from viral replicase systems in vitro. Methods for isolating double-stranded RNA include treatment with phenol and detergent, which remove proteins from nucleic acids; under such conditions complementary strands tend to become double-stranded. Double-stranded RNA is indicated by resistance to ribonuclease and, after sufficient purification and concentration, by biochemical characterization or examination with the electron microscope. Arguments against the existence of double-stranded RNA include experiments showing that very little ribonuclease-resistant RNA is found in infected tissue extracts or replicase systems when they are not treated with phenol or detergent. An immunofluorescent technique was used to suggest the presence of double-stranded RNA in the cytoplasm of cells infected with sindbis virus (*2*). Since as few as five nucleotide pairs may be sufficient to provide a binding site for antibodies, this technique is

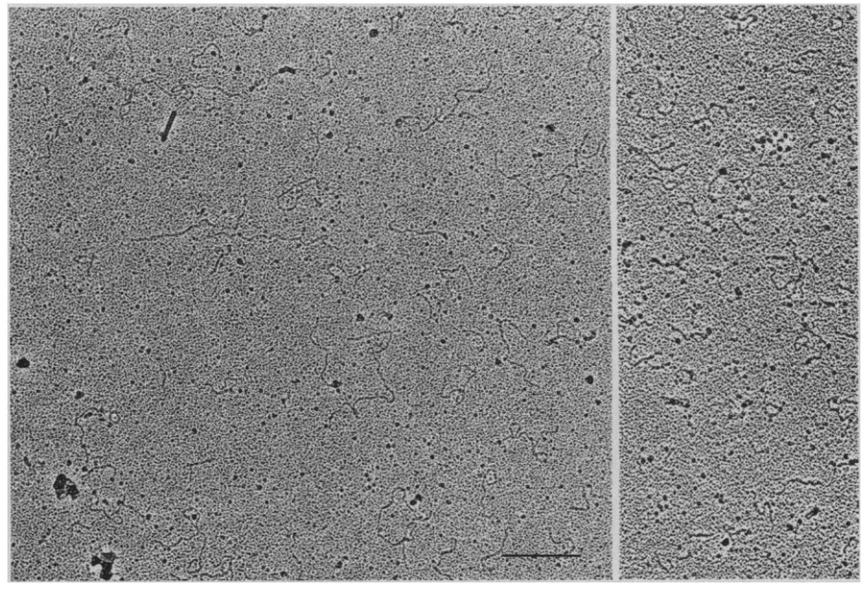

Fig. 1. Electron micrographs showing double-stranded RNA from an extract of tobacco leaf infected with TMV (left) and from a solution containing double-stranded polyinosinic · polycytidylic acid (2 μg/ml) (right). Samples of tobacco leaves inoculated with TMV were collected at intervals after inoculation and stored at −20°C. Double-stranded RNA was readily detected 40 hours after inoculation. The result shown is from a sample collected 4 days after inoculation. Filmed electron microscope grids were floated for 30 minutes on antiserum to double-stranded RNA (diluted 1 : 1000 with 0.05*M* tris-HCl, *p*H 7.2). The grids were washed with this tris buffer and placed on dilutions of synthetic double-stranded RNA, or on extracts of leaf tissue prepared by grinding 60 mg of frozen tissue with 0.54 ml of the tris buffer containing 0.4*M* sucrose and 0.15*M* NaCl (*10*). After 2 hours, the grids were washed with the tris buffer and placed on cytochrome c (0.1 mg/ml) in tris buffer for 15 minutes. The grids were then washed with distilled water, dipped for 30 seconds in $5 \times 10^{-5}M$ uranyl acetate and $5 \times 10^{-5}M$ HCl in 95 percent ethanol, rinsed with 95 percent ethanol, dried in air, and rotary-shadowed with platinum-palladium (*5*) (scale bar, 500 nm).

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not useful for distinguishing between short regions of base-pairing and double-stranded RNA of high molecular weight (*2*).

Using serologically specific electron microscopy (*3*), I have been able to assay extracts of virus-infected tissue for double-stranded RNA, without prior purification or the use of phenol or detergent. Electron microscope grids with Parlodion-carbon films were floated on dilute antiserum to double-stranded RNA (*4*) for 30 minutes. During this time a layer of serum proteins was adsorbed to the film. Excess serum was removed by washing, and the grids were placed on tissue extracts or RNA preparations for 2 hours. If double-stranded RNA was present, it was specifically attached to the grid by reacting serologically with the adsorbed antiserum. The other components in the extract were removed by washing, and the double-stranded RNA was stained and rotary-shadowed (*5*) for examination with the electron microscope. Double-stranded RNA was readily detected in extracts of tobacco infected with tobacco mosaic virus (TMV) and, for comparison, in a solution of synthetic double-stranded RNA (Fig. 1). Double-stranded RNA was not found in control experiments using normal serum or antiserums to several different plant viruses and infected tobacco plants, or antiserum to double-stranded RNA and mock-inoculated or healthy tobacco plants.

Measurements of the molecules on several negatives, similar to the one used for Fig. 1 (left), indicated a number of long molecules, 1.5 to 1.7 μm in length, and some that were considerably shorter (Fig. 2). This variation in length is consistent with observations made on double-stranded RNA isolated from TMV-infected tissue (*6*). The long molecules compare favorably with the calculated length of 1.67 μm for the double-stranded form of the complete genome of TMV (*7*).

Antiserums produced to synthetic double-stranded RNA have been shown to react to a limited extent with DNA-RNA hybrids (*8*). The antiserum to double-stranded RNA used in this study does not react with ribosomal RNA, single-stranded RNA, or DNA (*4*); it has not been tested with DNA-RNA hybrids. There are several reasons for believing that the filamentous molecules observed in extracts of tobacco leaves inoculated with TMV are indeed double-stranded RNA associated with virus replication and not DNA-RNA hybrids: (i) The molecules are not observed in extracts of healthy or mock-inoculated tobacco leaves. (ii) They are present in relatively high concentrations a few days after inoculation with TMV, which is a time of rapid virus synthesis. They are present in low concentrations in older infections of systematically infected leaves where the rate of virus synthesis is low. (iii) The size of the molecules is as expected for double-stranded RNA associated with TMV replication.

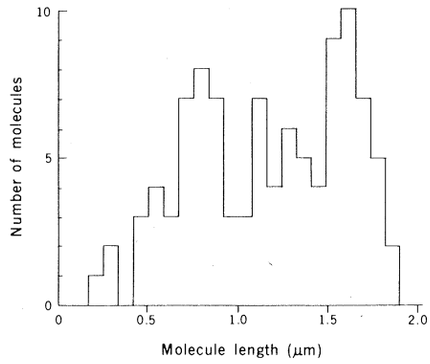

Fig. 2. Length distributions of double-stranded RNA from TMV-infected tobacco leaf. Electron microscope negatives were projected, and 100 linear molecules without overlaps of branching were selected at random and measured with a map measurer.

This technique appears to have several potential applications. In addition to demonstrating the existence of native double-stranded RNA, it may be useful in determining the concentration (*9*) and size of double-stranded forms as they occur in time-course experiments, and in studying infectious agents of unknown etiology that are suspected of being RNA viruses.

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References and Notes

1. For discussion of this problem see A. Gibbs and *B. Harrison* [*Plant Virology* (Wiley, New York, 1976), pp. 140–142] and H. Fraenkel-Conrat [*The Chemistry and Biology of Viruses* (Academic Press, New York, 1969), pp. 189–202].
2. B. Stollar and V. Stollar, *Virology* **42**, 276 (1970).
3. K. Derrick and R. Brlansky, *Phytopathology* **66**, 815 (1976).
4. I thank R. M. Lister for antiserum specific to double-stranded RNA. The serum was made against a complex of methylated bovine serum albumin and double-stranded polyinosinic · polycytidylic acid [E. Moffitt and R. Lister, *Phytopathology* **65**, 851 (1975)].
5. The staining and shadowing procedure for nucleic acids was used as described [R. Davis, M. Simon, N. Davidson, *Methods Enzymol.* **21D**, 413 (1971)].
6. A. Jackson, D. Mitchell, A. Siegel, *Virology* **45**, 182 (1971); R. Beachy and M. Zaitlin, *ibid.* **63**, 84 (1975).
7. This calculation was made using the predicted molecular weight of 4×10^6 daltons and the estimate of 2.4×10^6 dalton/μm for double-stranded RNA [J. Perrault, *Virology* **70**, 360 (1976)].
8. E. Schwartz and B. Stollar, *Biochem. Biophys. Res. Commun.* **35**, 115 (1969); B. D. Stollar, *Science* **169**, 609 (1970).
9. The technique has been used to make quantitative assays of plant viruses [K. Derrick, *Virology* **56**, 652 (1973)].
10. Assays for double-stranded RNA can be done with extracts prepared with tris buffer. The addition of sucrose and NaCl decreases the amount of debris on the grids.

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Sodium Requirement for the Positive Inotropic Action of Isoproterenol on Guinea Pig Atria

Abstract. *Isoproterenol does not elicit its characteristic positive inotropic action in contracting guinea pig atria suspended in sodium-free media. However, the ability of isoproterenol to decrease the time to peak tension development during an individual contraction cycle is still present in sodium-free solutions. Removal of sodium diminished but did not eliminate the tissues' ability to elevate adenosine 3',5'-monophosphate in response to isoproterenol. The striking absence of an inotropic action by isoproterenol on atria in sodium-free media suggests that sodium (and possibly a sodium-calcium exchange across the sarcolemma) plays an important role in the inotropic action of catecholamines.*

The inotropic effects of isoproterenol and other beta-adrenergic agonists appear to be mediated by adenosine 3',5'-monophosphate (cyclic AMP) (*1*). Several aspects of cardiac calcium metabolism are affected by cyclic AMP. Isoproterenol, dibutyryl cyclic AMP, and the phosphodiesterase inhibitor 1-methyl-3-isobutylxanthine (MIX) influence the cardiac action potential in a manner which suggests that these agents increase the rate of transmembrane calcium influx during excitation (*2*). Cyclic AMP also enhances the phosphorylation of isolated cardiac sarcoplasmic reticulum (*3*) associated with stimulation of calcium-activated adenosinetriphosphatase activity and enhanced calcium uptake (*4*). Isoproterenol characteristically increases tension development and decreases time to peak tension. We now report that in sodium-free solutions, guinea pig atria respond to isoproterenol or MIX with a decrease in developed ten-

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